MECHANOCULTURE – Applications of dynamic pressure in manufacturing cell therapies

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INTRODUCTION: Mechanical forces regulate fate decisions in mesenchymal stem cells (MSCs) and musculoskeletal cells such as chondrocytes, yet these forces are not controlled during scale up manufacturing of cells for therapeutic use. We have developed a pulsed pneumatic system to apply dynamic hydrostatic pressures to cells grown in high-throughput manufacturing platforms such as spinner flasks. By employing the under-used gas phase of the culture environment, our objectives were to induce mechanotransduction in the cells in order to improve the effectiveness and consistency of bioreactor-grown stem cells and chondrocytes.

METHODS: We developed a series of bioreactors using commercially available components and adapted these to our application using 3D printing. We constructed two devices: the J^2 bioreactor which is an evolution of our previously published design (Henstock *et al*, 2013; Reinwald *et al*, 2015) and uses a compressor and valve manifold to deliver pressures of up to 300kPa at 1Hz (fig. 1A). Our second bioreactor, the H^3 uses a simple motorized piston to compress the gas inside the culture environment (fig. 1B). In each bioreactor, we periodically subjected hMSC and chondrocytes in hydrogel constructs to dynamic pressures over the culture time and monitored the effects on proliferation, phenotype and extracellular matrix production using histochemical assays and µCT.

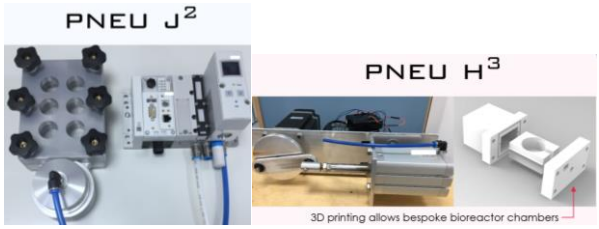

Figure 1. The J^2 bioreactor is an evolution of our previous design. The H^3 was developed as a student project and shown to be highly effective and manufactured at low cost from commercially available and 3D printed components.

RESULTS: Both bioreactors achieved their design parameters and maintained sterile cultures over several weeks. Example results show that chondrocytes cultured in alginate hydrogel microspheres increased their ECM production and proliferation rate (measured by MTT assay) after just 5 days exposure to dynamic pressures (fig. 2).

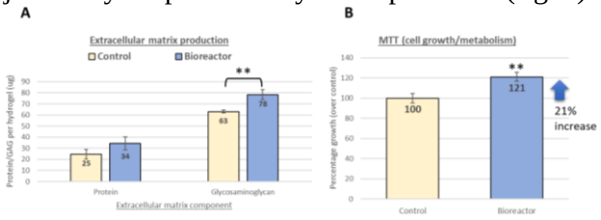

Figure 2. Biological effects of pulsed hydrostatic pressure on chondrocytes in hydrogels.

DISCUSSION & CONCLUSIONS: Mechanical stimulation is lacking in most scale up manufacturing platforms, yet we have shown that by simply pulse-compressing the gas phase in the culture vessel, pneumatic pressure is transduced into hydrostatic pressure in the media which is detected by cells as a form of compressive loading – recreating the forces that cells would experience in their native environment during exercise. We have therefore developed a novel 'mechano-culture' system that can be easily added to existing scale-up manufacturing platforms to pulse-compress the gas phase and deliver regulatory mechanical stimulation to cells in culture which helps maintain their phenotype during expansion.

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REFERENCES

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[2] Reinwald Y et al. Tissue Eng. C 2015;21:1-14